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(54) PIGRL-1, A MEMBER OF IMMUNOGLOBIN GENE SUPERFAMILY

(57) PIGRL-1 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing PIGRL-1 polypeptides and polynucleotides in the design of protocols for the treatment of Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D), among others and diagnostic assays for such conditions

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Description

[0001] This application claims the benefit of U S Provisional Application No 60/056 935 filed August 25 1997

FIELD OF INVENTION

[0002] This invention relates to newly identified polynucleotides polypeptides encoded by them and to the use of such polynucleotides and polypeptides and to their production More particularly the polynucleotides and polypeptides of the present invention relate to Immunoglobulin superfamily hereinafter referred to as PIGRL-1 The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides

BACKGROUND OF THE INVENTION

[0003] The immunoglobulin (Ig) gene superfamily comprises a large number of cell surface glycoproteins that share sequence homology with the V and C domains of antibody heavy and light chains These molecules function as receptors for antigens immunoglobulins and cytokines as well as adhesion molecules, and play important roles in regulating the complex cell interactions that occur within the immune system (A F Williams et al., Annu Rev Immuno 6 381-405. 1988, T Hunkapiller et al., Adv Immunol 44 1-63. 1989)

[0004] Several human immunodeficiency diseases derive from gene defects or from functional deregulation of the Ig superfamily proteins Examples are Hyper-IgM Immunodeficiency (HIM) caused by a defect in the gene encoding the ligand for CD40 (R C Allen et al Science 259 990-993. 1993). X-linked Severe Combined Immunodeficiency (XSCID) caused by mutations of the IL-2 receptor (M Noguchi et al Cell 73 147-157 1993) and IgA deficiency (IgA-D) linked to HLA-DQb (M A French et al., Immunol Today 11 271-274, 1990).

[0005] This indicates that these receptors have an established, proven history as therapeutic targets Clearly there is a need for identification and characterization of further receptors which can play a role in preventing ameliorating or correcting dysfunctions or diseases including but not limited to, Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID) and IgA deficiency (IgA-D)

SUMMARY OF THE INVENTION

[0006] In one aspect the invention relates to PIGRL-1 polypeptides and recombinant materials and methods for their production Another aspect of the invention relates to methods for using such PIGRL-1 polypeptides and polynucleotides Such uses include the treatment of Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D) among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with PIGRL-1 imbalance with the identified compounds Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate PIGRL-1 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

[0007] The following definitions are provided to facilitate understanding of certain terms used frequently herein

[0008] "PIGRL-1" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO 2, or an allelic variant thereof.

[0009] "Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said PIGRL-1 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said PIGRL-1.

[0010] "PIGRL-1 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO 1 or allelic variants thereof and/or their complements.

[0011] "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library

[0012] "Isolated" means altered "by the hand of man" from the natural state If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated" as the term is employed herein

[0013] "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodi-

fied RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

[0014] "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

[0015] "Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

[0016] "Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g., (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequenc-

es include, but are not limited to those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

[0017] As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[0018] Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

[0019] In one aspect, the present invention relates to PIGRL-1 polypeptides (or PIGRL-1 proteins). The PIGRL-1 polypeptides include the polypeptides of SEQ ID NOS:2 and 4, as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2, and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within PIGRL-1 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably PIGRL-1 polypeptides exhibit at least one biological activity of the receptor.

[0020] The PIGRL-1 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

[0021] Fragments of the PIGRL-1 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned PIGRL-1 polypeptides. As with PIGRL-1 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of PIGRL-1 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

[0022] Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of PIGRL-1 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in

an animal, especially in a human.

[0023] Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

[0024] The PIGRL-1 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

[0025] Another aspect of the invention relates to PIGRL-1 polynucleotides. PIGRL-1 polynucleotides include isolated polynucleotides which encode the PIGRL-1 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, PIGRL-1 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a PIGRL-1 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. PIGRL-1 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the PIGRL-1 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under PIGRL-1 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such PIGRL-1 polynucleotides.

[0026] PIGRL-1 of the invention is structurally related to other proteins of the Immunoglobulin superfamily, as shown by the results of sequencing the cDNA encoding human PIGRL-1. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 146 to 1315) encoding a polypeptide of 390 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 41.51% identity (using BLASTX) in 53 amino acid residues with Mouse polymeric immunoglobulin receptor (J. F. Piskurich et al., J. Immunol. 150:1735-1747, 1995). Furthermore, PIGRL-1 is 38.18% identical to human polymeric immunoglobulin receptor over 55 amino acid residues (P. Krajci et al., Eur. J. Immunol. 22:2309-2315, 1992). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 66.25% identity (using BLASTN) in 80 nucleotide residues with Rana catesbeiana myosin II (C. F. Solc et al., Aud. Neurosci. 1:63-75, 1994). Thus, PIGRL-1 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

```
   1  ACGAGCCTCA TCGTCAAGCT TTGTTCCTCG TGGGGGCTAG AAATCTCTTT
  51  CCAGTTCCAG ATTGTGAAGG GTTCCTGAGT AAGCAGCGTG TCTCCATCCC
 101  CCTCTCTAGG GGCTCTTGGA TGGACCTTGC ACTCTAGAAG GGACAATGGA
 151  GTTCTGGCTT TGGCCACTTT ACTTCCTGCC AGTATCAGGG GCCCTGAGGA
 201  TCCTCCCAGA AGTAAAGGTA GAGGGGGAGC TGGGCGGATC AGTTACCATC
 251  AAGTGCCCAC TTCCTGAAAT GCATGTGAGG ATATATCTGT GCCGGGAGAT
 301  GGCTGGATCT GGAACATGTG GTACCGTGGT ATCCACCACC AACTTCATCA
 351  AGGCAGAATA CAAGGGCCGA GTTACTCTGA AGCAATACCC ACGCAAGAAT
 401  CTGTTCCTAG TGGAGGTAAC ACAGCTGACA GAAAGTGACA GCGGAGTCTA
 451  TGCCTGCGGA GCGGGCATGA ACACAGACCG GGGAAAGACC CAGAAAGTCA
 501  CCCTGAATGT CCACAGTGAA TACGAGCCAT CATGGGAAGA GCAGCCAATG
 551  CCTGAGACTC CAAAATGGTT TCATCTGCCC TATTTGTTCC AGATGCCTGC
 601  ATATGCCAGT TCTTCCAAAT TCGTAACCAG AGTTACCACA CCAGCTCAAA
 651  GGGGCAAGGT CCCTCCAGTT CACCACTCCT CCCCCACCAC CCAAATCACC
 701  CACCGCCCTC GAGTGTCCAG AGCATCTTCA GTAGCAGGTG ACAAGCCCCC
 751  AACCTTCCTG CCATCCACTA CAGCCTCAAA AATCTCAGCT CTGGAGGGGC
 801  TGCTCAAGCC CCAGACGCCC AGCTACAACC ACCACACCAG GCTGCACAGG
 851  CAGAGAGCAC TGGACTATGG CTCACAGTCT GGGAGGGAAG GCCAAGGATT
 901  TCACATCCTG ATCCCGACCA TCCTGGGCCT TTTCCTGCTG GCACTTCTGG
 951  GGCTGGTGGT GAAAAGGGCC GTTGAAAGGA GGAAAGCCCT CTCCAGGCGG
1001  GCCCGCCGAC TGGCCGTGAG GATGCGCGCC CTGGAGAGCT CCCAGAGGCC
1051  CCGCGGGTCG CCGCGACCGC GCTCCCAAAA CAACATCTAC AGCGCCTGCC
1101  CGCGGCGCGC TCGTGGAGCG GACGCTGCAG GCACAGGGGA GGCCCCCGTT
1151  CCCGGCCCCG GAGCGCCGTT GCCCCCCGCC CCGCTGCAGG TGTCTGAATC
```

[a] A nucleotide sequence of a human PIGRL 1 (SEQ ID NO: 1).

Table 2[b]

```
   1  MDFWLWPLYF LPVSGALRIL PEVKVEGELG GSVTIKCPLP EMHVRIYLCR

  51  EMAGSGTCGT VVSTTNFIKA EYKGRVTLKQ YPRKNLFLVE VTQLTESDSG

 101  VYACGAGMNT DRGKTQKVTL NVHSEYEPSW EEQPMPETPK WFHLPYLFQM

 151  PAYASSSKFV TRVTTPAQRG KVPPVHHSSP TTQITHRPRV SRASSVAGDK

 201  PRTFLPSTTA SKISALEGLL KPQTPSYNHH TRLHRQRALD YGSQSGREGQ

 251  GFHILIPTIL GLFLLALLGL VVKRAVERRK ALSRPARRLA VRMRALESSQ

 301  RPRGSPRPRS QNNIYSACPR RARGADAAGT GEAPVPGPGA PLPPAPLQVS

 351  ESPWLHAPSL KTSCEYVSLY HQPAAMMEDS DSDDYINVPA
```

[b] An amino acid sequence of a human PIGRL-1 (SEQ ID NO: 2).

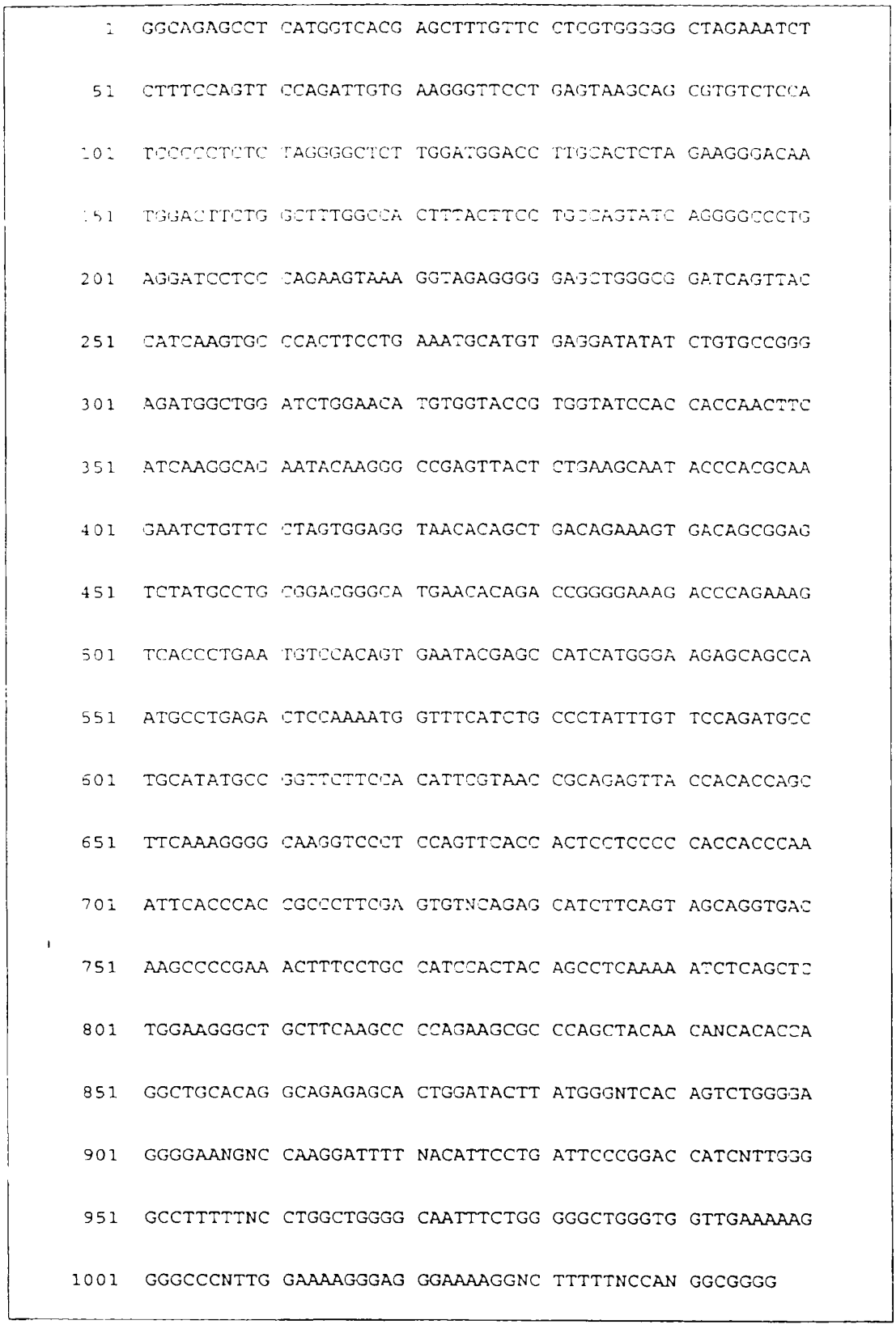
[0027] One polynucleotide of the present invention encoding PIGRL-1 may be obtained using standard cloning and screening from a cDNA library derived from mRNA in cells of human fetal heart, tonsils, bone marrow and leukocytes using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) *355:632-634*; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

[0028] The nucleotide sequence encoding PIGRL-1 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 146 to 1315 of SEQ ID NO:1), or it may be a sequence which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

[0029] When the polynucleotides of the invention are used for the recombinant production of PIGRL-1 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

[0030] Further preferred embodiments are polynucleotides encoding PIGRL-1 variants comprising the amino acid sequence of PIGRL-1 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3[c]



```
   1  GGCAGAGCCT CATGGTCACG AGCTTTGTTC CTCGTGGGGG CTAGAAATCT
  51  CTTTCCAGTT CCAGATTGTG AAGGGTTCCT GAGTAAGCAG CGTGTCTCCA
 101  TCCCCCTCTC TAGGGGCTCT TGGATGGACC TTGCACTCTA GAAGGGACAA
 151  TGGACTTCTG GCTTTGGCCA CTTTACTTCC TGCCAGTATC AGGGGCCCTG
 201  AGGATCCTCC CAGAAGTAAA GGTAGAGGGG GAGCTGGGCG GATCAGTTAC
 251  CATCAAGTGC CCACTTCCTG AAATGCATGT GAGGATATAT CTGTGCCGGG
 301  AGATGGCTGG ATCTGGAACA TGTGGTACCG TGGTATCCAC CACCAACTTC
 351  ATCAAGGCAC AATACAAGGG CCGAGTTACT CTGAAGCAAT ACCCACGCAA
 401  GAATCTGTTC CTAGTGGAGG TAACACAGCT GACAGAAAGT GACAGCGGAG
 451  TCTATGCCTG CGGACGGGCA TGAACACAGA CCGGGGAAAG ACCCAGAAAG
 501  TCACCCTGAA TGTCCACAGT GAATACGAGC CATCATGGGA AGAGCAGCCA
 551  ATGCCTGAGA CTCCAAAATG GTTTCATCTG CCCTATTTGT TCCAGATGCC
 601  TGCATATGCC GGTTCTTCCA CATTCGTAAC CGCAGAGTTA CCACACCAGC
 651  TTCAAAGGGG CAAGGTCCCT CCAGTTCACC ACTCCTCCCC CACCACCCAA
 701  ATTCACCCAC CGCCCTTCGA GTGTNCAGAG CATCTTCAGT AGCAGGTGAC
 751  AAGCCCCGAA ACTTTCCTGC CATCCACTAC AGCCTCAAAA ATCTCAGCTC
 801  TGGAAGGGCT GCTTCAAGCC CCAGAAGCGC CCAGCTACAA CANCACACCA
 851  GGCTGCACAG GCAGAGAGCA CTGGATACTT ATGGGNTCAC AGTCTGGGGA
 901  GGGGAANGNC CAAGGATTTT NACATTCCTG ATTCCCGGAC CATCNTTGGG
 951  GCCTTTTTNC CTGGCTGGGG CAATTTCTGG GGGCTGGGTG GTTGAAAAAG
1001  GGGCCCNTTG GAAAAGGGAG GGAAAAGGNC TTTTTNCCAN GGCGGGG
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A partial nucleotide sequence of a human PIGRL-1 (SEQ ID NO: 3).

Table 4[d]

```
  1  MDFWLWPLYF LPVSGALRIL PEVKVEGELG GSVTIKCPLP EMHVRIYLCR

 51  EMAGSGTCGT VVSTTNFIKA EYKGRVTLKQ YPRKNLFLVE VTQLTESDSG

101  VYACGRA
```

[d] A partial amino acid sequence of a human PIGRL-1 (SEQ ID NO: 4).

[0031] The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

[0032] Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO 1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding PIGRL-1 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the PIGRL-1 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

[0033] In one embodiment, to obtain a polynucleotide encoding PIGRL-1 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO 3) and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

[0034] The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

[0035] The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

[0036] For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

[0037] Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

[0038] A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as bac-

uloviruses papova viruses such as SV40 vaccinia viruses adenoviruses fowl pox viruses pseudorabies viruses and retroviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra)*.

[0039] For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

[0040] If the PIGRL-1 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If PIGRL-1 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

[0041] PIGRL-1 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

[0042] This invention also relates to the use of PIGRL-1 polynucleotides for use as diagnostic reagents. Detection of a mutated form of PIGRL-1 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of PIGRL-1. Individuals carrying mutations in the PIGRL-1 gene may be detected at the DNA level by a variety of techniques.

[0043] Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled PIGRL-1 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al., Science* (1985) 230 1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al., Proc Natl Acad Sci USA* (1985) 85 4397-440}. In another embodiment, an array of oligonucleotides probes comprising PIGRL-1 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

[0044] The diagnostic assays offer a process for diagnosing or determining a susceptibility to Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D) through detection of mutation in the PIGRL-1 gene by the methods described.

[0045] In addition, Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D), can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of PIGRL-1 polypeptide or PIGRL-1 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an PIGRL-1, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

[0046] Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D), which comprises:

(a) a PIGRL-1 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
(b) a nucleotide sequence complementary to that of (a);
(c) a PIGRL-1 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
(d) an antibody to a PIGRL-1 polypeptide, preferably to the polypeptide of SEQ ID NO: 2. It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

[0047] The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

[0048] The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the PIGRL-1 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

[0049] Antibodies generated against the PIGRL-1 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

[0050] Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

[0051] The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

[0052] Antibodies against PIGRL-1 polypeptides may also be employed to treat Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D), among others.

Vaccines

[0053] Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with PIGRL-1 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D), among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering PIGRL-1 polypeptide via a vector directing expression of PIGRL-1 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

[0054] Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a PIGRL-1 polypeptide wherein the composition comprises a PIGRL-1 polypeptide or PIGRL-1 gene. The vaccine formulation may further comprise a suitable carrier. Since PIGRL-1 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The for-

mulations may be presented in unit-dose or multi-dose containers for example sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation such as oil-in water systems and other systems known in the art The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation

Screening Assays

[0055] The PIGRL-1 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention Thus polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in for example cells cell-free preparations chemical libraries and natural product mixtures These substrates and ligands : : be natural substrates and ligands or may be structural or functional mimetics See Coligan *et al.*, *Current Protocc.. n Immunology* 1(2) Chapter 5 (1991)

[0056] PIGRL-1 polypeptides are responsible for many biological functions, including many pathologies. Accordingly it is desirous to find compounds and drugs which stimulate PIGRL-1 on the one hand and which can inhibit the function of PIGRL-1 on the other hand. In general agonists are employed for therapeutic and prophylactic purposes for such conditions as Hyper-IgM Immunodeficiency (HIM) X-linked Severe Combined Immunodeficiency (XSCID) and IgA deficiency (IgA-D) Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as Hyper-IgM Immunodeficiency (HIM) X-linked Severe Combined Immunodeficiency (XSCID) and IgA deficiency (IgA-D)

[0057] In general such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof Such cells include cells from mammals yeast *Drosophila* or *E. coli* Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding or stimulation or inhibition of a functional response

[0058] The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor Further these assays may test whether the candidate compound results in a signal generated by activation of the receptor using detection systems appropriate to the cells bearing the receptor at their surfaces Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed

[0059] Further the assays may simply comprise the steps of mixing a candidate compound with a solution containing a PIGRL-1 polypeptide to form a mixture measuring PIGRL-1 activity in the mixture and comparing the PIGRL-1 activity of the mixture to a standard

[0060] The PIGRL-1 cDNA protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of PIGRL-1 mRNA and protein in cells For example an ELISA may be constructed for measuring secreted or cell associated levels of PIGRL-1 protein using monoclonal and polyclonal antibodies by standard methods known in the art and this can be used to discover agents which may inhibit or enhance the production of PIGRL-1 (also called antagonist or agonist respectively) from suitably manipulated cells or tissues Standard methods for conducting screening assays are well understood in the art

[0061] Examples of potential PIGRL-1 antagonists include antibodies or in some cases oligonucleotides or proteins which are closely related to the ligand of the PIGRL-1 e.g., a fragment of the ligand or small molecules which bind to the receptor but do not elicit a response so that the activity of the receptor is prevented

[0062] Thus in another aspect the present invention relates to a screening kit for identifying agonists antagonists ligands receptors substrates enzymes etc for PIGRL-1 polypeptides or compounds which decrease or enhance the production of PIGRL-1 polypeptides which comprises:

(a) a PIGRL-1 polypeptide preferably that of SEQ ID NO 2;
(b) a recombinant cell expressing a PIGRL-1 polypeptide preferably that of SEQ ID NO 2;
(c) a cell membrane expressing a PIGRL-1 polypeptide preferably that of SEQ ID NO: 2; or
(d) antibody to a PIGRL-1 polypeptide preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit (a) (b) (c) or (d) may comprise a substantial component

Prophylactic and Therapeutic Methods

[0063] This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of PIGRL-1 activity

[0064] If the activity of PIGRL-1 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the PIGRL-1, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

[0065] In another approach, soluble forms of PIGRL-1 polypeptides still capable of binding the ligand in competition with endogenous PIGRL-1 may be administered. Typical embodiments of such competitors comprise fragments of the PIGRL-1 polypeptide.

[0066] In still another approach, expression of the gene encoding endogenous PIGRL-1 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56: 560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6: 3073; Cooney *et al.*, *Science* (1988) 241: 456; Dervan *et al.*, *Science* (1991) 251: 1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

[0067] For treating abnormal conditions related to an under-expression of PIGRL-1 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates PIGRL-1, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of PIGRL-1 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

[0068] Peptides, such as the soluble form of PIGRL-1 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

[0069] Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

[0070] Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

[0071] The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

[0072] Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

[0073] The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

[0074] While there are several methods to obtain the full length cDNA, two are outlined below.

1) The method of Rapid Amplification of cDNA Ends (RACE) can be utilized to obtain the 5' end. See Frohman et al., Proc. Nat. Acad. Sci. USA 85, 8998-9002, (1988). Briefly, specific oligonucleotides are annealed to mRNA and used to prime the synthesis of the cDNA strand. Following destruction of the mRNA with RNaseH, a poly C anchor sequence is added to the 3' end of the cDNA and the resulting fragment is amplified using a nested set of antisense primers and an anchor sequence primer. The amplified fragment is cloned into an appropriate vector and subjected to restriction and sequence analysis.

2) The polymerase chain reaction can be used to amplify the 5' end of the cDNA from human cDNA libraries using sequential rounds of nested PCR with two sets of primers. One set of antisense primers is specific to the 5' end of the partial cDNA and the other set of primers anneals to a vector specific sequence. The amplified products are cloned into an appropriate vector and subjected to restriction and sequence analysis.

Example 2, PIGRL-1 belongs to immunoglobulin (Ig) superfamily.

[0075] PIGRL-1 is a new member of the Ig family. The predicted partial protein sequence of this new gene shows modest, but extended, homology to polymeric Ig receptor (pIgR). The pIgR plays a crucial role in mucosal immunity by translocating polymeric IgA and IgM through secretory epithelial cells into external body fluids (J. P. Kraehenbuhl et al., Physiol. Rev. 72 853-879, 1992), an important process in defense against the invasion of microbial pathogens. Based on the multiple tissue dot blot and northern blot data, Expression of PIGRL-1 is restricted to the immune system, suggesting a role in immune function and a candidate for drug targeting.

[0076] The extracellular region of PIGRL-1 contains a single Ig domain with a V-like fold as shown by (1) the presence of Ig V fold conserved residues and (2) homology to several other Ig like proteins (poly Ig V1 and V4, CMRF35, TCR Vβ and Ig κ V_L).

[0077] In the following alignment, dashes indicate positions where residues are identical to the V1 region of the poly Ig receptor (poly Ig RV1). Residues in poly Ig RV1 that are highly conserved in Ig variable regions (A. N. Barclay et al., The leukocyte antigen facts book, 2nd edition, Academic Press, 1997) are shown in bold.

```
                    B                 C           C'
PIGRL-1     pevk-ege l g--r k-p l-...  em-v -i-l- -ema qs-t... g      42 (SEQ ID NO 4)
CMRF35   lsh mt agp v g l -q r -eke. h-t l n----  r-p qilr . -dk   45 (SEQ ID NO 5)
PolyIgRV4     prs tv kg- a s -a-l-p nrk..esks i----l-ega qn-r...-pl   45 (SEQ ID NO 6)
Ig κ VL   tqt as-eva v-qt t k-q asqsis...- yls--qqk-- q-pk-li-..   45 (SEQ ID NO 7)
TCR Vβ   sqk-srciicq r-t-lt-q-q v.dsq ..  mm- -rq--- qslt-iatan   46 (SEQ ID NO 8)
PolyIgRV1   ---------- ---------- -------- -----.--.- ----. ..-
Consensus   IFGPEEVNSV EGNSVSITCY YPPTSVNRHT RKYWC-RQPG ARGGL--CIT   47 (SEQ ID NO 9)

                C''           D          E                  F
PIGRL-1     v--ttn--ka e-k--vt-kq --rknl-l-e -t--tes--  v-a--a-m-t   92 (SEQ ID NO 4)
CMRF35   i-etk-sa.g -rn--vsird s-a-ls-t-t len-te--a- t-w--vdtpw   92 (SEQ ID NO 5)
PolyIgRV4   --d-----ka q-e--ls-le e-g- --t-i ln--tsr-a- f-w-ltngdt   95 (SEQ ID NO 6)
Ig κ VL   .rast-a-g .vss-fkgsg .sgref tlt -sgveca-aa t-y-qq-ws-   92 (SEQ ID NO 7)
TCR Vβ   qg-eat e g fvidkfpisr -nlrfsnlr -sn--p---s i-l-sve.ge   94 (SEQ ID NO 8)
PolyIgRV1   ---------- ---------- ---------- ---------- - -  ..
Consensus   LISSEGYVSS KYAGRANLTN FPENGTFVVN IAQLSQDDSG RYKCGLGINS   97 (SEQ ID NO 9)

PIGRL-1     d--ktqk-t- n-  (SEQ ID NO: 4)
CMRF35      --d-.h-piv --  (SEQ ID NO: 5)
PolyIgRV4   --....rttv --  (SEQ ID NO: 6)
Ig κ VL     snvenvfg.. ..  (SEQ ID NO: 7)
TCR Vβ   agdtq-fgp. ..  (SEQ ID NO: 8)
PolyIgRV1   .          -
Consensus   LRGLSFDVSL EV  (SEQ ID NO: 9)
```

Example 3. PIGRL-1 gene expression pattern:

[0078] PIGRL-1, a new member of the Ig superfamily, has been identified. The predicted protein sequence of this new gene shows modest, but extended, homology to polymeric Ig receptor family proteins, particularly in the extracellular domain. Based on the Clontech's Human RNA Master Blot and Multiple Tissue Northern Blot results, PIGRL-1 is exclusively expressed in spleen, thymus, lymph nodes and peripheral leukocytes, suggesting a role in immune function. Thus, this protein is a candidate target for diseases of the immune system such as Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D).

Example 4. Recombinant soluble PIGRL-1 proteins.

[0079] The extracellular domain of PIGRL-1 is expressed as a secreted soluble protein by truncation at the start of the transmembrane domain (glycine 251 in Table 2) as has been described for other immunoglobulin domain proteins, e.g. for CD4 (K. C. Deen et al., Nature 331:82-84 (1988)). PIGRL-1 is also expressed as a secreted, soluble Ig fusion protein by linking the same extracellular region of PIGRL-1 to the hinge and constant domains of heavy chain IgG such as has been described for CD4 (D. J. Capon et al., Nature 317: 525-531 (1989)). In addition, preparation of oligomeric Ig fusion proteins is possible by addition of the tailpiece segment of IgM or IgA to the C-terminus of the Fc domain of IgGs, as exemplified for the IgM tailpiece segment in R. I. F. Smith and S. L. Morrison, Biotechnology 12: 683-688 (1994) and in R. I. F. Smith, et al., J. Immunol. 154: 2226-2236 (1995). These proteins are produced in insect cells or

in mammalian cells such as COS-7 or CHO purified by standard methodology and are useful as tool therapeutic and diagnostic agents. Thus these proteins are used to

a) Determine the cleavage site of the N-terminal leader by amino acid sequence analysis of this processed recombinant protein

b) Prepare polyclonal and monoclonal antibodies for

1) Detection of PIGRL-1 protein expression in different tissues and cell types

2) Functional studies of PIGRL-1 protein such as induction of cell differentiation and proliferation cytokine production and cell death assays

c) Test for agonist/antagonist activity when added to cultured cells and in animal models of immune disease

d) Search for its ligand(s)

e) Establish screen assays for small molecule agonists or antagonists of PIGRL-1 protein which may be potential therapeutic and/or diagnostic agents

[0080] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth

Annex to the description

[0081]

1) GENERAL INFORMATION

(i) APPLICANT
(A) NAME: SMITHKLINE BEECHAM CORPORATION
(B) STREET: ONE FRANKLIN PLAZA
(C) CITY: PHILADELPHIA
(D) STATE OR PROVINCE: PENNSYLVANIA
(E) COUNTRY: USA
(F) POSTAL CODE: 19103

(ii) TITLE OF THE INVENTION: PIGR1-1, A MEMBER OF IMMUNOGLOBULIN GENE SUPERFAMILY

(iii) NUMBER OF SEQUENCES: 9

(iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2040 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACGAGCCTCA TCGTCAAGCT TTGTTCCTCG TGGGGGCTAG AAATCTCTTT CCAGTTCCAG 60
ATTGTGAAGG GTTCCTGAGT AAGCAGCGTG TCTCCATCCC CCTCTCTAGG GGCTCTTGGA 120
TGGACCTTGC ACTCTAGAAG GGACAATGGA CTTCTGGCTT TGGCCACTTT ACTTCCTGCC 180
AGTATCAGGG GCCCTGAGGA TCCTCCCAGA AGTAAAGGTA GAGGGGAGC TGGGCGGATC 240
AGTTACCATC AAGTGCCCAC TTCCTGAAAT GCATGTGAGG ATATATCTGT GCCGGGAGAT 300
GGCTGGATCT GGAACATGTG GTACCGTGGT ATCCACCACC AACTTCATCA AGGCAGAATA 360
CAAGGGCCGA GTTACTCTGA AGCAATACCC ACGCAAGAAT CTGTTCCTAG TGGAGGTAAC 420
ACAGCTGACA GAAAGTGACA GCGGAGTCTA TGCCTGCGGA GCGGGCATGA ACACAGACCG 480
GGGAAAGACC CAGAAAGTCA CCCTGAATGT CCACAGTGAA TACGAGCCAT CATGGGAAGA 540
GCAGGCAATG CCTGAGACTC CAAAATGGTT TCATCTGCCC TATTTGTTCC AGATGCCTGC 600
ATATGCCAGT TCTTCCAAAT TCGTAACCAG AGTTACCACA CCAGCTCAAA GGGGCAAGGT 660
CCCTCCAGTT CACCACTCCT CCCCCACCAC CCAAATCACC CACCGCCCTC GAGTGTCCAG 720
AGCATCTTCA GTAGCAGGTG ACAAGCCCCG AACCTTCCTG CCATCCACTA CAGCCTCAAA 780
AATCTCAGCT CTGGAGGGGC TGCTCAAGCC CCAGACGCCC AGCTACAACC ACCACACCAG 840

```
GCTGCACAGG CAGAGAGCAC TGGACTATGG CTCACAGTCT GGGAGGGAAG GCCAAGGATT     900
TCACATCCTG ATCCCGACCA TCCTGGGCCT TTTCCTGCTG GCACTTCTGG GGCTGGTGGT     960
GAAAAGGGCC GTTGAAAGGA GGAAAGCCCT CTCCAGGCGG GCCCGCCGAC TGGCCGTGAG    1020
GATGCGGCGC CTGGAGAGCT CCCAGAGGCC CCGCGGGTCG CCGCGACCGC GCTCCCAAAA    1080
TAACATCTAC AGCGCCTGCC CGCGGGCGGC TCGTGGAGGG GACGCTGCAG GCACAGGGGA    1140
TGCTCCGGTT CCTCGCCCTG GAGCGCCGTT GCGGCGCGCC CCGCTGGAGT TGTCTGAATT    1200
TCCCTGGCTC GATGCCCCAT CTCTGAAGAC CAGCTGTGAA TACGTGAGCT TCTACCACCA    1260
GCCTGCCGCG ATGATGGAGG ACAGTGATTC AGATGACTAC ATCAATGTTC CTGCCTGACA    1320
ACTCCCCAGC TATGCCCCAA CCCCAGGCTC GGACTGTGGT GCCAAGGAGT CTCATCTATC    1380
TGCTGATGTC GAATACCTGC TTCATGTGTT CTCAGAGCCC TCATCACTTC CCATGCCCCA    1440
TCTCGACTCC CATCCCCATC TATCTGTGCC CTGAGCATGG CTCTGCCCCC AGGTCGTCTT    1500
GCACACCTTG GCAGCCCCCT GTAGTTGACA GGTAAGCTGT AGGCATGTAG AGCAATTGTC    1560
CCAATGCCAC TTGCTTCCTT TCCAAGCCGT CGAACAGACT GTGGGATTTG CAGAGTGTTT    1620
CTTCCATGTC TTTGACCACA GGGTTGTTGC TGCCCAGGCT CTAGATCACA TGGCATCAGG    1680
CTGGGGCAGA GGCATAGCTA TTGTCTCGGG CATCCCTTCC CAGGGTTGGG TCTTACACAA    1740
ATAGAAGGCT CTTGCTCTGA GTTATGTGAC ATGCCTCAGC CCCATGGACT AAGCAGGGGT    1800
CTGGTATAAA AACACTCCTG GAAACGCCTT TGCCCTGATC CAAATGTTAG CACTTGCTAG    1860
TGAACGTCTA CTTATCTCAA GTTCTATGCT AAAGGCAATT TATCTTGATG TGATGATAAA    1920
CCAAACTTAT TAGCAAGATA TGCATATATA TCCATAAATT CTCTTTACTC TGTCTCCATC    1980
ACTTGATGCA CATAAGTGCC CTGACCTCAG CATCTCCCCT CTAAAAAAAA AAAAAAAAAA    2040
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 390 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Phe Trp Leu Trp Pro Leu Tyr Phe Leu Pro Val Ser Gly Ala
 1               5                  10                  15
Leu Arg Ile Leu Pro Glu Val Lys Val Glu Gly Glu Leu Gly Gly Ser
            20                  25                  30
Val Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu
        35                  40                  45
Cys Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr
    50                  55                  60
Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln
65                  70                  75                  80
```

Tyr Pro Arg Lys Asn Leu Phe Leu Val Glu Val Thr Gln Leu Thr Glu
85 90 95

Ser Asp Ser Gly Val Tyr Ala Cys Gly Ala Gly Met Asn Thr Asp Arg
100 105 110

Gly Lys Thr Gln Lys Val Thr Leu Asn Val His Ser Glu Tyr Glu Pro
115 120 125

Ser Trp Glu Glu Gln Pro Met Pro Glu Thr Pro Lys Trp Phe His Leu
130 135 140

Pro Tyr Leu Phe Gln Met Pro Ala Tyr Ala Ser Ser Ser Lys Phe Val
145 150 155 160

Thr Arg Val Thr Thr Pro Ala Gln Arg Gly Lys Val Pro Pro Val His
165 170 175

His Ser Ser Pro Thr Thr Gln Ile Thr His Arg Pro Arg Val Ser Arg
180 185 190

Ala Ser Ser Val Ala Gly Asp Lys Pro Arg Thr Phe Leu Pro Ser Thr
195 200 205

Thr Ala Ser Lys Ile Ser Ala Leu Glu Gly Leu Leu Lys Pro Gln Thr
210 215 220

Pro Ser Tyr Asn His His Thr Arg Leu His Arg Gln Arg Ala Leu Asp
225 230 235 240

Tyr Gly Ser Gln Ser Gly Arg Glu Gly Gln Gly Phe His Ile Leu Ile
245 250 255

Pro Thr Ile Leu Gly Leu Phe Leu Leu Ala Leu Leu Gly Leu Val Val
260 265 270

Lys Arg Ala Val Glu Arg Arg Lys Ala Leu Ser Arg Arg Ala Arg Arg
275 280 285

Leu Ala Val Arg Met Arg Ala Leu Glu Ser Ser Gln Arg Pro Arg Gly
290 295 300

Ser Pro Arg Pro Arg Ser Gln Asn Asn Ile Tyr Ser Ala Cys Pro Arg
305 310 315 320

Arg Ala Arg Gly Ala Asp Ala Ala Gly Thr Gly Glu Ala Pro Val Pro
325 330 335

Gly Pro Gly Ala Pro Leu Pro Pro Ala Pro Leu Gln Val Ser Glu Ser
340 345 350

Pro Trp Leu His Ala Pro Ser Leu Lys Thr Ser Cys Glu Tyr Val Ser
355 360 365

Leu Tyr His Gln Pro Ala Ala Met Met Glu Asp Ser Asp Ser Asp Asp
370 375 380

Tyr Ile Asn Val Pro Ala
385 390

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1047 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGCACAGCCT CATGGTCACG AGCTTTGTTC CTCGTGGGGG CTAGAAATCT CTTTCCAGTT      60
CCAGATTGTG AAGGGTTCCT GAGTAAGCAG CGTGTCTCCA TCCCCCTCTC TAGGGGCTCT     120
TGGATGGACC TTGCACTCTA GAAGGGACAA TGGACTTCTG GCTTTGGCCA CTTTACTTCC     180
TGCCAGTACC AGGGGCCCTG AGGATCCTCC CAGAAGTAAA GGTAGAGGGG GAGCTGGGCC     240
GATCAGTTAC CATCAAGTGC CCACTTCCTG AAATGCATGT GAGGATATAT CTGTGCCGGG     300
AGATGGCTGG ATCCGGAACA TGTGGTACCG TGGTATCCAC CACCAACTTC ATCAAGGCAG     360
AACACAAGGC CGGAGTTACT CTGAAGCAAT ACCCACGCAA GAATCTGTTC CTAGTGGAGG     420
TAACACAGTT GACAGAAAGT GACAGCGGAG TCTATGCCTG CGGACGGGCA TGAACACAGA     480
CCGGGAAAAG ACCCAGAAAG TCACCCTGAA TGTCCACAGT GAATACGACC CATCATGGGA     540
AGAGCAGCCA ATGCCTGAGA CTCCAAAATT GTTTCATCTG CCCTATTTGT TCCACATGCC     600
TGCATATGGC GGTTCTTCCA CATTCGTAAC CGCAGAGTTA CCACACCAGC TTCAAAGGGG     660
CAAGGTCCCT CCAGTTCACC ACTCCTCCCC CACCACCCAA ATTCACCCAC CGCCCTTCGA     720
GTGTNCAGAA CATCTTCAGT ASCAGGTGAC AAGCCCCGAA ACTTTCCTGC CATCCACTAC     780
AGCCTCAAAA ATCTCAGCTC TGGAAGGGCT GCTTCAAGCC CCAGAAGCGC CCAGCTACAA     840
CANCACACCA GGCTGCACAG GCAGAGACCA CTGGATACTT ATGGGNTCAC AGTCTGGGGA     900
CGGGAANGNC CAAGGATTTT NACATTCCTG ATTCCCGGAC CATCNTTGGG GCCTTTTTNC     960
CTGGCTGGGG CAATTTCTGG GGGCTGGGTG GTTGAAAAAG GGGCCCNTTG GAAAAGGGAG    1020
GGAAAAGGNC TTTTTNCCAN GGCGGGG                                        1047
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Asp Phe Trp Leu Trp Pro Leu Tyr Phe Leu Pro Val Ser Gly Ala
 1               5                  10                  15
```

Leu Arg Ile Leu Pro Glu Val Lys Val Gl. Gly Glu Leu Gly Gly Ser
20 25 30
Val Thr Ile Lys Cys Pro Leu Pro Glu Met His Val Arg Ile Tyr Leu
35 40 45
Cys Arg Glu Met Ala Gly Ser Gly Thr Cys Gly Thr Val Val Ser Thr
50 55 60
Thr Asn Phe Ile Lys Ala Glu Tyr Lys Gly Arg Val Thr Leu Lys Gln
65 70 75 80
Tyr Pro Arg Lys Asn Leu Phe Leu Val G.. Val Thr Gln Leu Thr Glu
85 90 95
Ser Asp Ser Gly Val Tyr Ala Cys Gly Arg Ala
100 105

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Ser His Met Thr Ala Gly Pro Val Gly Leu Gln Arg Glu Lys Glu
1 5 10 15
His Thr Leu Asn Arg Pro Gln Ile Leu Arg Asp Lys Ile Glu Thr Lys
20 25 30
Ser Ala Gly Arg Asn Val Ser Ile Arg Asp Ser Ala Leu Ser Thr Thr
35 40 45
Leu Glu Asn Thr Glu Ala Thr Trp Val Asp Thr Pro Trp Asp His Pro
50 55 60
Ile Val
65

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Pro Arg Ser Thr Val Lys Gly Ala Ser Ala Leu Pro Asn Arg Lys Glu
 1               5                  10                  15
Ser Lys Ser Ile Leu Ala Gly Ala Gln Asn Arg Pro Leu Asp Lys Ala
            20                  25                  30
Gln Glu Leu Ser Leu Glu Thr Gly Thr Ile Asn Leu Thr Ser Arg Ala
        35                  40                  45
Phe Trp Leu Thr Asn Gly Asp Thr Arg Thr Thr Val
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Thr Gln Thr Ala Ser Glu Val Ala Val Gly Thr Thr Lys Gln Ala Ser
 1               5                  10                  15
Gln Ser Ile Ser Tyr Leu Ser Gln Gln Lys Gln Pro Lys Leu Ile Arg
            20                  25                  30
Ala Ser Thr Ala Gly Val Ser Ser Phe Lys Gly Ser Gly Ser Gly Thr
        35                  40                  45
Glu Phe Thr Leu Thr Ser Gly Val Glu Cys Ala Ala Ala Thr Tyr Gln
    50                  55                  60
Gln Trp Ser Ser Asn Val Glu Asn Val Phe Gly
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ser Gln Lys Ser Arg Asp Ile Cys Gln Arg Thr Leu Thr Gln Gln Val
 1               5                  10                  15
Asp Ser Gln Met Met Arg Gln Gln Ser Leu Thr Ile Ala Thr Ala Asn
            20                  25                  30
Gly Gly Glu Ala Thr Glu Gly Phe Val Ile Asp Lys Phe Pro Ile Ser
        35                  40                  45
Arg Asn Leu Thr Phe Ser Thr Leu Thr Ser Asn Pro Ser Ile Leu Ser
    50                  55                  60
Val Glu Gly Glu Ala Gly Asp Thr Gln Phe Gly Pro
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Ile Phe Gly Pro Glu Glu Val Asn Ser Val Glu Gly Asn Ser Val Ser
 1               5                  10                  15
Ile Thr Cys Tyr Tyr Pro Pro Thr Ser Val Asn Arg His Thr Arg Lys
            20                  25                  30
Tyr Trp Cys Arg Gln Pro Gly Ala Arg Gly Gly Leu Cys Ile Thr Leu
        35                  40                  45
Ile Ser Ser Glu Gly Tyr Val Ser Ser Lys Tyr Ala Gly Arg Ala Asn
    50                  55                  60
Leu Thr Asn Phe Pro Glu Asn Gly Thr Phe Val Val Asn Ile Ala Gln
65                  70                  75                  80
Leu Ser Gln Asp Asp Ser Gly Arg Tyr Lys Cys Gly Leu Gly Ile Asn
                85                  90                  95
Ser Leu Arg Gly Leu Ser Phe Asp Val Ser Leu Glu Val
            100                 105
```

Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the PIGRL-1 polypeptide of SEQ ID NO 2 or a nucleotide sequence complementary to said isolated polynucleotide

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO 1 encoding the PIGRL-1 polypeptide of SEQ ID NO2

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO 1 over its entire length

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO 1

5. The polynucleotide of claim 1 which is DNA or RNA

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a PIGRL-1 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO 2 when said expression system is present in a compatible host cell

7. A host cell comprising the expression system of claim 6

8. A process for producing a PIGRL-1 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture

9. A process for producing a cell which produces a PIGRL-1 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a PIGRL-1 polypeptide

10. A PIGRL-1 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO 2 over its entire length

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO 2

12. An antibody immunospecific for the PIGRL-1 polypeptide of claim 10

13. A method for the treatment of a subject in need of enhanced activity or expression of PIGRL-1 polypeptide of claim 10 comprising

    (a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or
    (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the PIGRL-1 polypeptide of SEQ ID NO 2 over its entire length, or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said receptor activity *in vivo*

14. A method for the treatment of a subject having need to inhibit activity or expression of PIGRL-1 polypeptide of claim 10 comprising

    (a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or
    (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor, and/or
    (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of PIGRL-1 polypeptide of claim 10 in a subject comprising

    (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said PIGRL-1

polypeptide in the genome of said subject and/or
(b) analyzing for the presence or amount of the PIGRL-1 polypeptide expression in a sample derived from said subject

16. A method for identifying agonists to PIGRL-1 polypeptide of claim 10 comprising

(a) contacting a cell which produces a PIGRL-1 polypeptide with a candidate compound and
(b) determining whether the candidate compound effects a signal generated by activation of the PIGRL-1 polypeptide

17. An agonist identified by the method of claim 16

18. The method for identifying antagonists to PIGRL-1 polypeptide of claim 10 comprising

(a) contacting a cell which produces a PIGRL-1 polypeptide with an agonist and
(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound

19. An antagonist identified by the method of claim 18

20. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a PIGRL-1 polypeptide

Office européen des brevets (11) EP 0 905 238 A3

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(54) PIGRL-1, A MEMBER OF IMMUNOGLOBIN GENE SUPERFAMILY

(57) PIGRL-1 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing PIGRL-1 polypeptides and polynucleotides in the design of protocols for the treatment of Hyper-IgM Immunodeficiency (HIM), X-linked Severe Combined Immunodeficiency (XSCID), and IgA deficiency (IgA-D), among others and diagnostic assays for such conditions.

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European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 30 6487

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl.6)
P,X	HITOSHI Y ET AL: "Toso, a cell surface, specific regulator of Fas-induced apoptosis in T cells." IMMUNITY, (1998 APR) 8 (4) 461-71. JOURNAL CODE: CCF. ISSN: 1074-7613.. XP002100823 United States * figure 1 *	1-11,20	C12N15/13 C07K14/705 C07K16/28 A61K38/17

TECHNICAL FIELDS SEARCHED (Int Cl.6)

C12N
C07K
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely

Claims searched incompletely

Claims not searched

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	18 May 1999	Espen, J

CATEGORY OF CITED DOCUMENTS

X particularly relevant if taken alone
Y particularly relevant if combined with another document of the same category
A technological background
O non-written disclosure
P intermediate document

T theory or principle underlying the invention
E earlier patent document, but published on, or after the filing date
D document cited in the application
L document cited for other reasons

& member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C03)

Although claims 13,14 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched completely:
1-13,15,16,18,20

Claim(s) searched incompletely:
14,17,19

Reason for the limitation of the search:

Said claims relate to agonists/antagonists without giving a true technical characterization of the claimed matter. In consequence, the socpe of said claims is ahiguous and, moreover, their subject-matter is vague and not sufficiently disclosed.